

Claims

1. A method for the construction of randomized gene libraries in suitable cells comprising the following steps

introducing into cells capable of homologous recombination

10 a) a target vector comprising a first DNA sequence coding for at least a γ -subunit of a *Kluyveromyces lactis* killer toxin as negative selection marker, said DNA sequence being flanked at its 5' end by a first target sequence and at its 3' end by a second target sequence and

15 b) a donor DNA sequence which is flanked at its 5' end by a DNA sequence which is homologous to said first target sequence and flanked at its 3' end by a DNA sequence which is homologous to said second target sequence and

20 cultivation of said cells under suitable conditions allowing the selection of cells in which said DNA sequence in the target vector encoding at least a γ -subunit of a *Kluyveromyces lactis* killer toxin has been replaced by said donor sequence by means of homologous recombination thereby abolishing expression of said γ -subunit of a *K. lactis* killer toxin.

25 2. The method of claim 1, wherein said target vector further comprises a second DNA sequence encoding at least one protein region, preferably more than two protein regions, more preferably a full length protein.

30 3. The method of claim 2 wherein said first DNA sequence of said target vector encoding at least the γ -subunit of the *K. lactis* killer toxin and being flanked by said two target sequences replaces a protein region encoding DNA sequence of said second DNA sequence comprised in said target vector.

4. The method of claims 1 to 3 wherein said DNA sequence encoding at least the γ subunit of the *K. lactis* killer toxin is under control of a heterologous promoter, preferably a constitutive promoter, more preferably a *TEF* promoter from *Ashbya gossypii*.

5 5. The method of claim 4 wherein said promoter is located between the DNA sequence encoding at least the γ subunit of *K. lactis* killer toxin and one of the two target sequences.

10 6. The method of claims 1 to 5, wherein said first DNA sequence of said target vector comprises at least one unique recognition site for a restriction enzyme.

15 7. The method of claim 6, wherein said unique recognition site is located in the coding region of the γ -toxin DNA sequence or preferably between the coding region of the γ -toxin DNA sequence and the promoter.

20 8. The method of claims 1 to 7 wherein said second DNA sequence encodes an antibody or a single chain antibody.

25 9. The method of claim 8 wherein said first DNA sequence of said target vector replaces at least one CDR region of said antibody or said single chain antibody, preferably a CDR3V_L region, more preferably a CDR2 and a CDR3 region.

10. The method of claims 8 or 9 wherein said first DNA sequence comprising the γ subunit of *K. lactis* killer toxin is transcribed in the opposite direction of said antibody or single chain antibody gene.

30 11. The method of claims 1 to 10 wherein said γ -toxin subunit of the *K. lactis* killer toxin lacks the signal peptide.

35 12. The method of claims 1 to 11 wherein said host cells are yeast cells, preferably *Saccharomyces cerevisiae* cells.

13. The method of claims 1 to 12 wherein said target vector is introduced into said host cells in linearized form.

14. The method of claim 13 wherein said target vector is linearized by cutting with a restriction enzyme recognizing in said first DNA sequence of said target vector said at least one unique recognition site.

15. The method of claims 1 to 14 wherein said donor sequence comprises a DNA sequence encoding a protein region, preferably a CDR region of an antibody.

16. The method of claims 1 to 15 wherein said target vector and said donor sequence are introduced into said host cells by co-transformation.

17. The method of claims 12 to 16 wherein said yeast cells are cultivated at a temperature selected from the range of 24°C to 30°C, preferably at 24°C.

18. Use of a *Kluyveromyces lactis* killer toxin, in particular a γ -subunit of said toxin, as negative selection marker for the construction of randomized gene libraries and/or region replacement by homologous recombination.

19. A DNA vector which comprises the following sequences: a first target sequence for homologous recombination, a TEF promoter from *Ashbya gossypii* driving transcription of a *K. lactis* killer toxin, a DNA sequence encoding at least a γ -subunit of a *K. lactis* killer toxin and a second target sequence for homologous recombination.

20. A host cell comprising a vector of claim 19, preferably a yeast cell, more preferably a *Saccharomyces cerevisiae* cell.